

HUMAN-PATHOGENIC FUNGI IN THE SOILS OF CENTRAL OHIO^{1, 2}

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ABSTRACT

A survey of soil from several sites in Franklin and surrounding counties in central Ohio for human-pathogenic fungi was undertaken. The samples were analyzed for the presence of dermatophytes and keratinophytic fungi by the hair-bait technique, for systemic mycotic agents by a direct spraying method, and for nocardiae by the paraffin-bait technique. The hair-bait technique revealed *Trichophyton ajelloi*, *Microsporum gypseum*, *M. cookei*, *Trichophyton terrestre*, *Chrysosporium tropicum*, *C. asperatum*, *C. keratinophilum*, *Arthroderma tuberculatum*, and *Ctenomyces serratus* in the soil of Ohio for the first time. Thirty-four strains of *Nocardia* were isolated by the paraffin-bait technique; 12 were specifically identified as *N. asteroides* by employing standard techniques. *Histoplasma capsulatum* and *Allescheria boydii* were isolated by direct spraying of the soil suspension onto selective media.

INTRODUCTION

In recent years much attention has been paid to studying human pathogenic fungi in their natural habitats. A knowledge of their presence in soil or in other habitats can explain the geographic distribution of a particular disease, which in turn will help in diagnosis and in prophylaxis. The presence of fungi pathogenic to man in natural environments, especially in soil, has been known, for most of the human-pathogenic fungi, for over a decade.

The development of several special techniques for the selective isolation of these fungi from the soil explains the progress made in this field. Among these methods is the hair-bait technique introduced by Vanbreuseghem (1952) for the isolation of keratinophilic fungi, including dermatophytes. This method was previously used in isolating keratinophilic chytrids by Karling (1946). Extensive surveys were conducted, using this technique, on the prevalence and distribution of geophilic dermatophytes and related keratinophilic fungi from all over the world. Some new keratinophilic fungi were reported first from soil, at least a few of them being shown later to be actual pathogens and most others being potential pathogens.

The intraperitoneal injection of a soil suspension treated with anti-bacterial antibiotics into a mouse, developed by Emmons (1949), contributed much to our knowledge of the natural habitat of the systemic mycotic agents. By using this method, *Histoplasma capsulatum* (Emmons, 1949), *Cryptococcus neoformans* (Emmons, 1951), and *Blastomyces dermatitidis* (Denton *et al.*, 1961) were isolated from soil. Direct streaking of the soil suspension onto media containing cycloheximide and antibacterial antibiotics isolated *Histoplasma capsulatum* and

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Coccidioides immitis. The selective paraffin-bait technique used for isolating *Nocardia* spp. by Gordon and Hagan (1936) has been employed widely throughout the world to study the prevalence and distribution of members of this genus in the soil.

No report on the prevalence in Ohio soils of fungi pathogenic for humans was found in the literature of the past twenty years. Hence a preliminary search for such fungi was undertaken. This paper reports the results of part of a project designed to study the prevalence of human-pathogenic fungi in the soil of Franklin and surrounding counties in Ohio, and the micro-ecological factors responsible for the growth of these organisms in the soil.

MATERIALS AND METHODS

Collection of soil samples

Soil samples were collected in sterile plastic bags by scooping up the uppermost two-inch layer of soil with a disposable plastic spoon, after removing the litter. The soil samples were taken from a variety of sites in and around Columbus, as follows: river bank samples from along the Olentangy River, south of the Lane Avenue bridge (5 samples) and north of the West North Broadway Avenue bridge (10); at the Columbus Zoo, in or around mammal enclosures (20) and in bird enclosures (10); at Blendon Woods Metropolitan Park east of Columbus (10); from a picnic area along the west side of the Scioto River below Griggs Dam (4); in an open field on The Ohio State University Farm, northwest of the farm buildings (5); from the opening and at arms-length in a rodent burrow along the Olentangy River, south of the Henderson Road bridge (5); from a cultivated field on The Ohio State University farm, last planted to soybean (5); and along a ridge, west of the Olentangy River, south of the Hayden Run bridge (3). Upon return to the laboratory, the soil samples were processed immediately or were stored at 4°C until they were studied. In most cases the samples were studied immediately for keratinophilic fungi.

Isolation of keratinophilic fungi

Sterile petri dishes 10 cm in diameter were half-filled with soil which was moistened with sterile distilled water; the amount of water added varied from sample to sample, depending on the moisture content of the sample. It was then baited with strands of autoclaved horse hair. After one or two weeks of incubation at room temperature, the growth of the hair was examined and cultured on Sabouraud's agar medium containing cycloheximide and chlortetracycline. Detailed morphology of each strain was studied and the fungi identified.

Isolation of Nocardia spp.

About five grams of the well-mixed soil were suspended in 10 to 15 ml of sterile distilled water, and the suspension was allowed to stand for 30 minutes. About one ml of the supernatant liquid was inoculated into a carbon-free broth of the following composition (McClung, 1960): sodium nitrate, 2 g; monobasic potassium phosphate, 0.008 g; manganese chloride, 0.002 g; distilled water, 1000 ml; pH, 7.2. The broth was sterilized by autoclaving at 121°C for 20 minutes. Paraffin-coated glass rods were prepared and sterilized according to the method described by Kurup, Randhawa, and Sandhu (1968), and one rod each was aseptically introduced into the carbon-free broth inoculated with the soil suspension. The baited soil suspensions were incubated at 37°C for up to 15 days. *Nocardia* spp. usually appeared on the paraffin bait in seven to ten days as brown, yellow, pink, or white tuft-like growths. Growth from each tuft was made into a suspension and streaked onto glucose nutrient agar plates. Suspect colonies were identified to species.

Isolation of mycotic agents by direct streaking

To isolate mycotic agents, a modified method of Swatek, Wilson, and Omieczynski (1967) was followed. About 10 grams of the well-mixed soil was suspended in 90 ml of sterile distilled water and shaken vigorously for two minutes. The suspension was allowed to stand for one hour. The supernatant fluid was further diluted with sterile distilled water and the suspension atomized over Sabouraud's agar, Littman's medium with chlortetracycline, and Sabouraud's agar with cycloheximide and chlortetracycline in triplicate for each dilution. Usually 10^{-3} , 10^{-4} , or 10^{-5} dilutions were used as inoculum. The inoculated plates were incubated at room temperature for up to two weeks. Suspected colonies were subcultured and the organisms identified.

RESULTS AND DISCUSSION

A total of 82 keratinophilic fungi were isolated from 59 of the 77 soil samples studies by the hair-bait technique (Table 1). The fungi represented 9 species belonging to 6 major keratinophilic genera. *Trichophyton ajelloi* was most prevalent, followed closely by *Microsporum gypseum*; *Microsporum cookei* was isolated

TABLE 1
Keratinophilic fungi recovered from different soil sites

	Zoo								
	River Bank	Animals	Birds	Park	Open field	Rodent Burrow	Cultivated Soil	Ridge	Total
No. of soil samples studied	15	20	10	14	5	5	5	3	77
No. negative	4	1	3	9	1	-	-	-	18
Organisms isolated									
<i>Microsporum gypseum</i>	1	15(3)	-	1	2(1)	-	-	1	20
<i>M. cookei</i>	-	1	-	-	-	-	-	-	1
<i>Trichophyton ajelloi</i>	9	2(1)	1(1)	3	2(1)	2	5(4)	1	25
<i>T. terrestre</i>	1	1	1	1	1	1	-	-	6
<i>Arthoderma tuberculatum</i>	1	-	2	-	-	-	-	-	3
<i>Chrysosporium tropicum</i>	3	2	4	-	2	1	-	-	12
<i>C. keratinophilum</i>	-	2	2	-	-	-	-	-	4
<i>C. asperatum</i>	-	1	-	1	-	-	1	1	4
<i>Ctenomyces serratus</i>	-	-	4	-	-	-	-	-	4
Unidentified	-	-	-	-	-	2	-	1	3

Number in parenthesis represents the number of strains that produced the perfect state.

only once. Thirty-four strains of *Nocardia* were isolated by the paraffin-bait technique. Of the 34 isolates, 12 proved to be *Nocardia asteroides* by standard criteria. All were partially acid-fast and were Gram positive. They failed to hydrolyze casein, tyrosine, xanthine, hypoxanthine, and adenine, but did hydrolyze uric acid. Acid was produced from glucose and glycerol. All hydrolyzed aesculin and grew at 45°C, survived for 8 hours at 50°C and showed resistance to lysozyme (Gordon and Horan, 1968). The remaining isolates have not yet been specifically identified. In addition, a large number of saprophytic *Mycobacterium* and *Streptomyces* spp. were recovered during the study.

One strain each of *Histoplasma capsulatum*, *Allescheria boydii*, *Trichophyton ajelloi*, *Microsporum cookei*, and *Chrysosporium tropicum* were isolated using the spraying method. All except *Allescheria boydii* were isolated in cycloheximide agar; this species was recovered on Sabouraud's agar.

Histoplasma capsulatum was isolated from a soil sample collected in October, 1968, from a vineyard at the University Farm, near Kenny Road and Lane Avenue. The colony appeared within a week on Sabouraud's agar with cycloheximide and chlortetracycline as a white, cottony growth. On subculture on Sabouraud's agar, it grew faster and produced smooth-walled, round-to-pyriform microconidia and a large number of tuberculate macroconidia, which were quite variable in size and shape. This isolate easily converted to its yeast form when cultured on brain-heart infusion agar incubated at 37°C for one to two weeks.

The single isolate of *Allescheria boydii* originated from a soil sample taken in November, 1968, from the Blendon Woods Metropolitan Park. The colony appeared on Sabouraud's agar as a greyish-white, cottony colony. Upon microscopic examination, large numbers of round-to-oval unicellular conidia were found attached to small undifferentiated conidiophores. Cleistothecia were produced in large numbers when the organism was transferred to corn meal agar.

The unusually high prevalence of keratinophilic fungi in soils of central Ohio is noteworthy. *Trichophyton ajelloi* was isolated from soil samples originating from all habitats in the present study (Table 1), a prevalence (32.5%) not comparable with the considerably lower percentage reported from surveys conducted by many investigators on this continent and from other parts of the world. The prevalence reported was very low in Italy (Ajello and Varsavsky, 1965), in Russia (Ganiev and Azimov, 1965), in Australia (Donald and Brown, 1962), in Argentina (Negroni *et al.*, 1964), in India (Randhawa and Sandhu, 1965) and even from other parts of North America (Dean and Haley, 1962; Al-Doory, 1967).

The results obtained with regard to the prevalence of *Microsporum gypsum* are comparable to the results obtained by other workers in this country and abroad. Over 75 percent of the *M. gypsum* isolates in the present study originated from soil samples collected at the Columbus Zoo. High prevalence of this species has been reported from places frequented by animals and by man. Similarly, the occurrence of *Trichophyton terrestre* in all the soil types studied is again in accord with the results of other investigators. All the four strains of *Ctenomyces serratus* originated from soil of avian habitats.

Seven of the 25 strains of *Trichophyton ajelloi* produced the perfect state, *Arthroderma uncinatum*, in the primary isolate. Similarly, four of 20 strains of *Microsporum gypsum* and one of six strains of *Trichophyton terrestre* produced *Nannizzia incurvata* and *Arthroderma quadrifidum*, respectively. The cleistothecia of *Arthroderma uncinatum* were globose, pale buff-colored, and measured 400 to 850 μ in diameter. Peridial hyphae were pale yellow in color and uncinately branched, usually to the outside of the main hypha. The cells were fairly thick walled, strongly echinulate, and dumbbell shaped. The cleistothecia of *Nannizzia incurvata* were globose, pale buff, and 350 to 700 μ in diameter. Peridial hyphae were pale buff, septate, branched, and densely echinulate. The inner cells of the peridial hyphae were not constricted and were often swollen towards the apices. The outer cells were symmetrically constricted with up to three constrictions. The secondary branches of the peridial hyphae invariably curved towards the main axis away from the cleistothecia; up to five branches arose in succession at the apex of the same cell. *Arthroderma quadrifidum* produced cleistothecia which were globose and pale buff, and measured 450 to 650 μ in diameter. Peridial hyphae were pale yellow, uncinately branched usually to the outside of the main hypha. Cells were thick walled, strongly echinulate, and dumbbell shaped when young, but when mature developed condyle-like appendages from one face only. Three other strains produced an *Arthroderma* perfect state, but they have not been identified specifically.

Nothing can be stated about the prevalence of the deep mycotic agents, mainly because the number of isolates is low, as is also the number of samples studied.

This preliminary survey reflects the high prevalence of keratinophilic fungi and *Nocardia* spp. in the soils of Ohio. A detailed survey covering many more

samples to ascertain the distribution of human-pathogenic fungi in central Ohio, with particular emphasis on the ecological factors determining the prevalence in various soil types and habitats, is essential before drawing any additional conclusions.

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